

SCANNING ELECTRON MICROSCOPY ON WING SCALES OF *COLIAS EURYTHEME*

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OPTICAL MICROSCOPY DISCLOSES that the scales on the wings of Lepidoptera may be ribbed lengthwise, with perpendicular cross-ribs to give a network (Gentil, 1935), but finer details cannot be resolved. Transmission electron microscopy has been utilized to study the fine structure of *Morpho* scales (Gentil, 1942; Kinder and Süffert, 1943; Richards, 1944), whose iridescent colors are "structural" and result from diffraction of light by ridges on the scale rather than from the presence of pigments. However, in the family Pieridae, including the genus *Colias*, the yellow and/or orange colors are not structural, and transmission electron microscopy has been reported to disclose round and spindle-shaped aggregations of pigment (Yagi, 1954), which consists of a number of pteridine compounds (Watt, 1964). The black scales in the border are colored by melanins.

The present work was undertaken on the premise that the recently-developed method of scanning electron microscopy (SEM) should be particularly well-suited, due to its advantageous magnification range (45-30,000 X) and depth of focus, to examination of the surface structure of the scales. *Colias eurytheme* (Boisduval) was chosen as an example. A particular object was to note possible variations in the fine structure of scales from different areas of the wing.

METHODS

Figure 1 shows a specimen, male, with indication of the areas examined. Small portions of these areas were cut out with a scalpel, and each was mounted on a specimen stub. The specimens were vapor-coated with a thin (300 Angstroms) layer of gold/palladium alloy (60/40) to render them conductive, a prerequisite for examination by SEM. The SEM instrument was a JEOLCO JSM-2, operated at an accelerating voltage of 25 kv. Photomicrographs were prepared with Polaroid P/N Type 55 film at a scan speed of 50 seconds per frame.

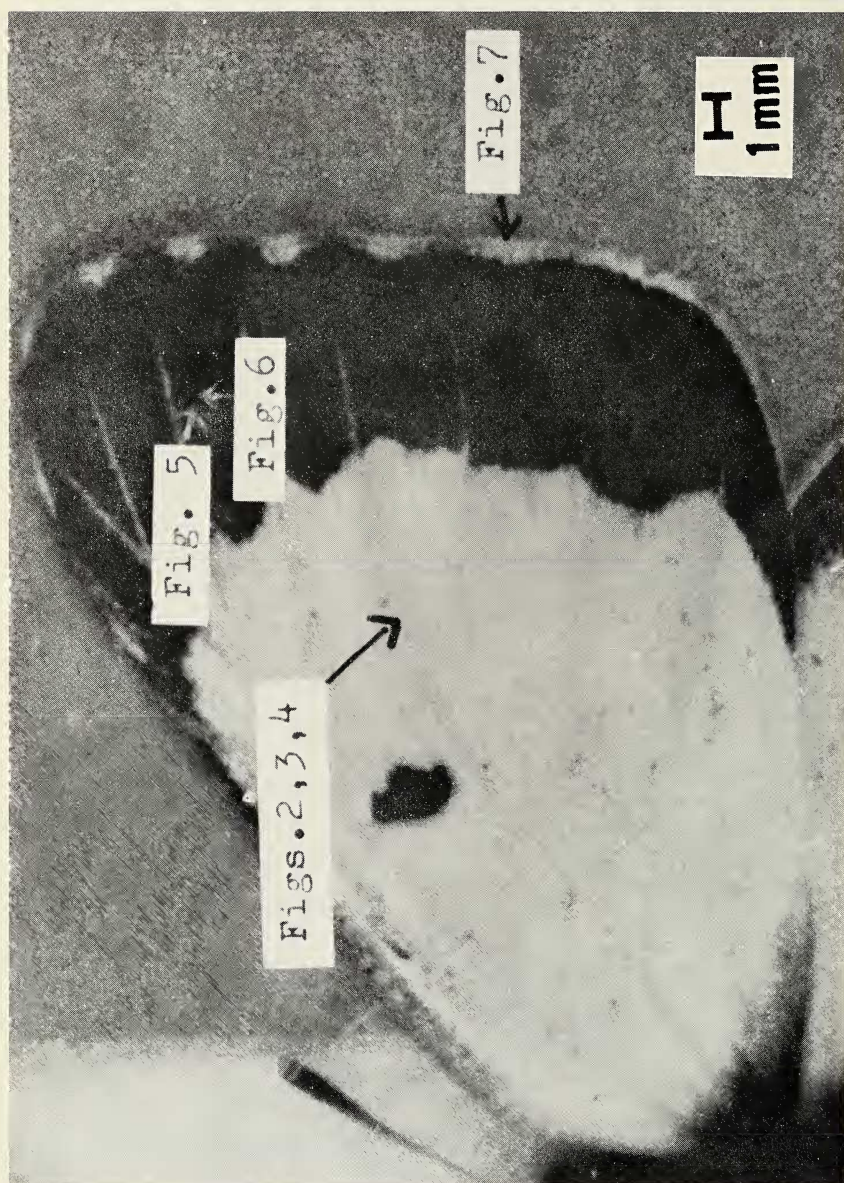


Fig. 1.—*Colias eurytheme*, male. Right forewing, showing location of scales examined.

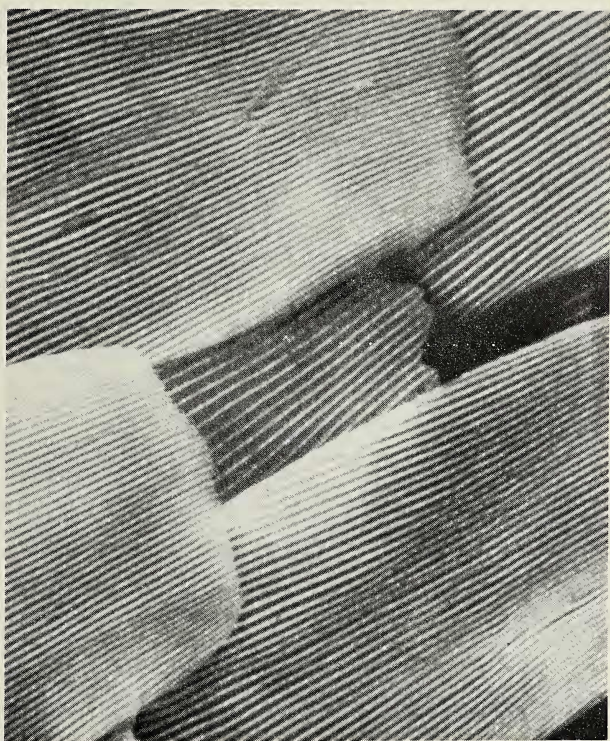
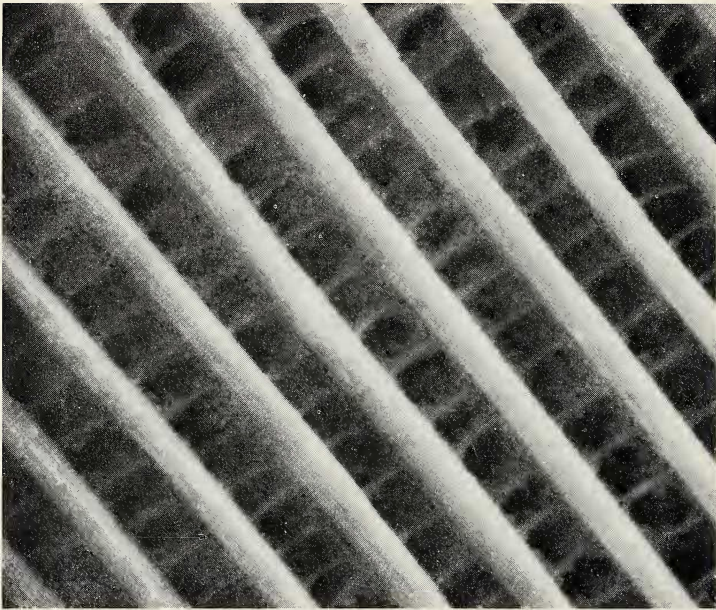
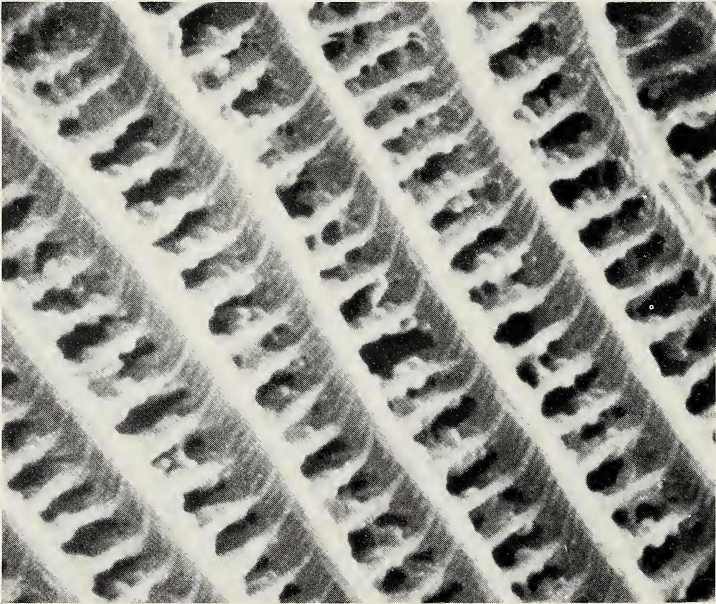


Fig. 2a.—Upper surface, orange scales.

To expose the underside of the scales, the wing was pressed onto a surface coated with contact adhesive (the backing used for Polaroid color prints), and the wing membrane was peeled off to leave the scales perfectly transferred. This method is successful because the peduncles (stems) are loosely held in sockets on the membrane, the scales of the upper and under layers being attached at alternating sockets (Gray, 1961).



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Figs. 2b, c.—Upper surface, orange scales.

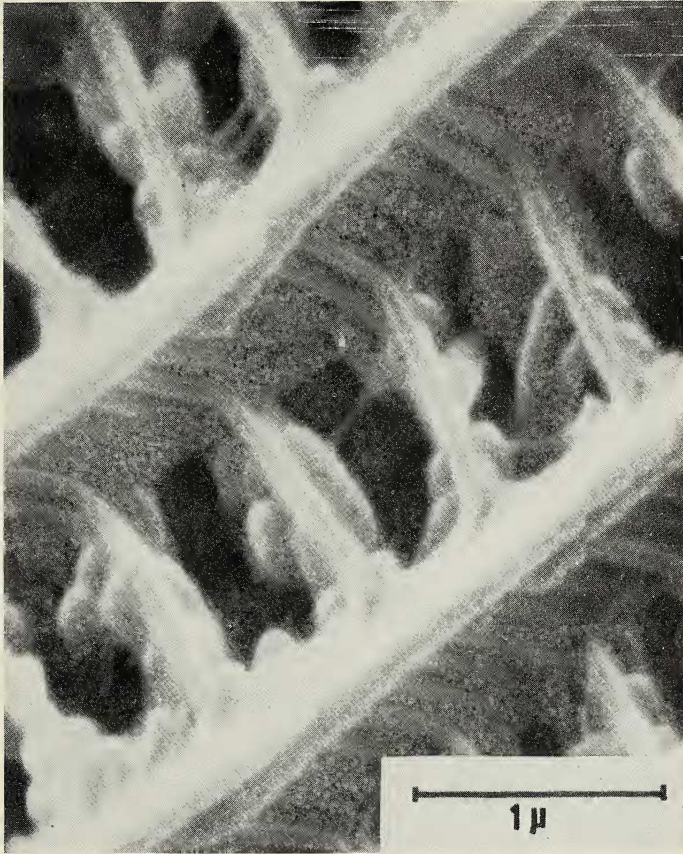


Fig. 2d.—Upper surface, orange scales.

OBSERVATIONS

Results are shown in Figs. 2-7. It is interesting that there are marked differences in fine structure among the four varieties of scales whose upper surfaces were examined and also between the upper and lower surfaces of the same (orange) scale. Butterfly scales long have been described as hollow, as suggested by the holes visible in the photomicrographs. The hollowness of the peduncle seems apparent in Figs. 4b and c. It has been speculated (Portier, 1932) that the scales and peduncles of the genus *Parnassius* are hollow and therefore admit air, communicate with tracheal capillaries in the wing, and play a role in respiration.

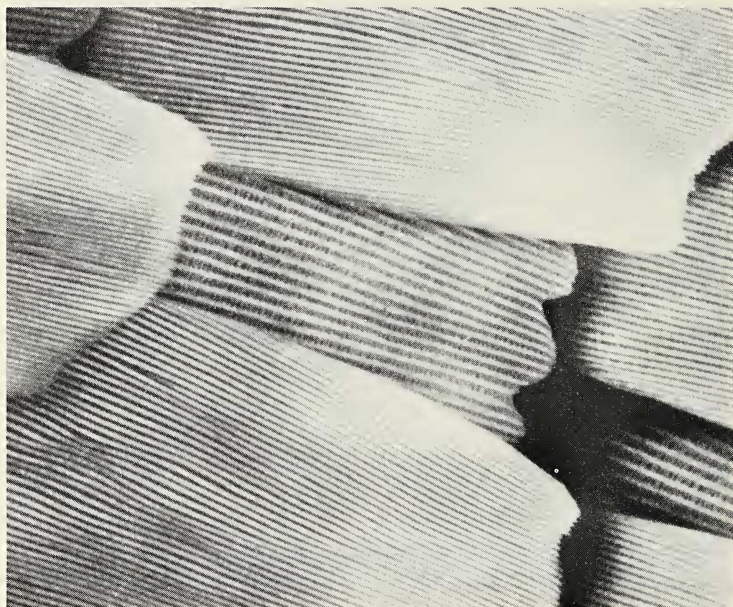
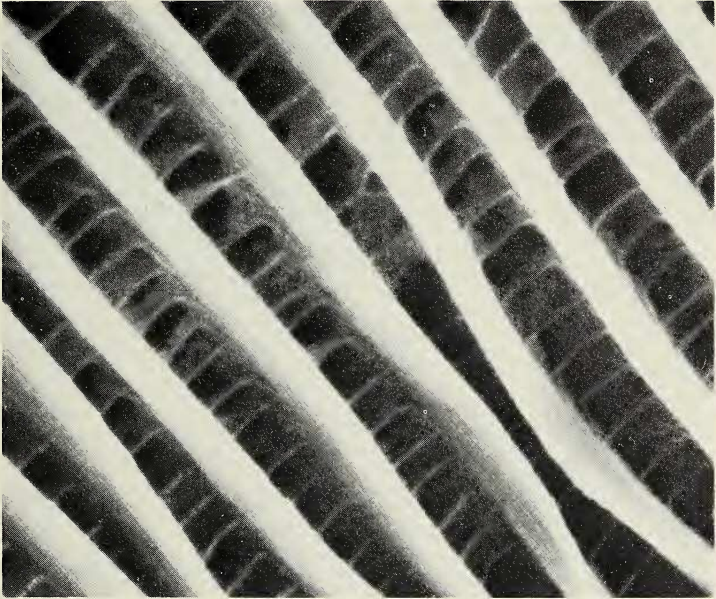
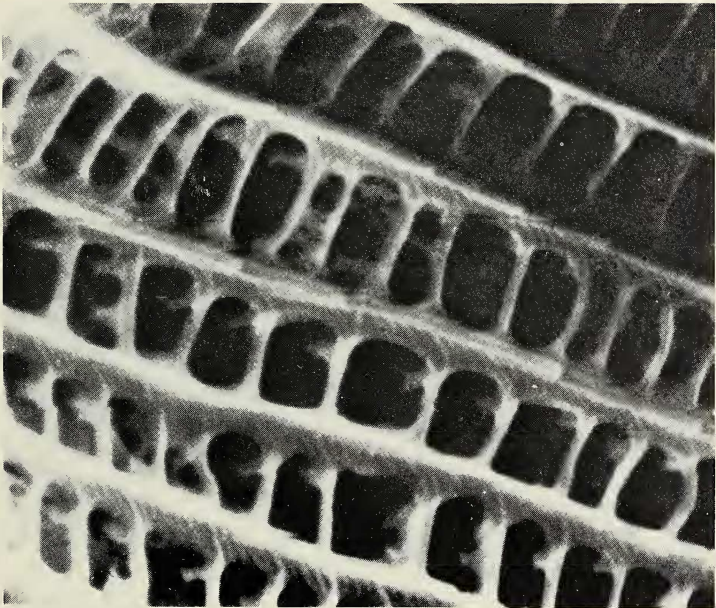


Fig. 3a.—Upper surface, washed orange scales.

The fine structure of the orange scales visible at 10,000X (Figs. 2b and c, 3b and c) resemble a "double grating" or network. The distance between the lengthwise ribs is approximately 1.5 microns, and that between the cross-ribs or connecting ribs ranges from approximately 0.5 to 0.7 micron. The thickness of the cross-ribs is approximately 0.07 micron. In some orange scales the cross-ribs appear to be partially interconnected by a thin skin or membrane (Fig. 2b) whereas in other orange scales most of the cross-ribs are not interconnected but exhibit small ellipsoidal structures that appear to be suspended from them (Figs. 2c and d). The above-described two types of orange scales are found in different positions with respect to the "shingling" arrangement (Fig. 2a) on the wing membrane; the scales with the ellipsoidal particles (Fig. 2c) occupy the lower layer and are partially covered by the upper-layer scales shown in Fig. 2b.



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Figs. 3b, c.—Upper surface, washed orange scales.

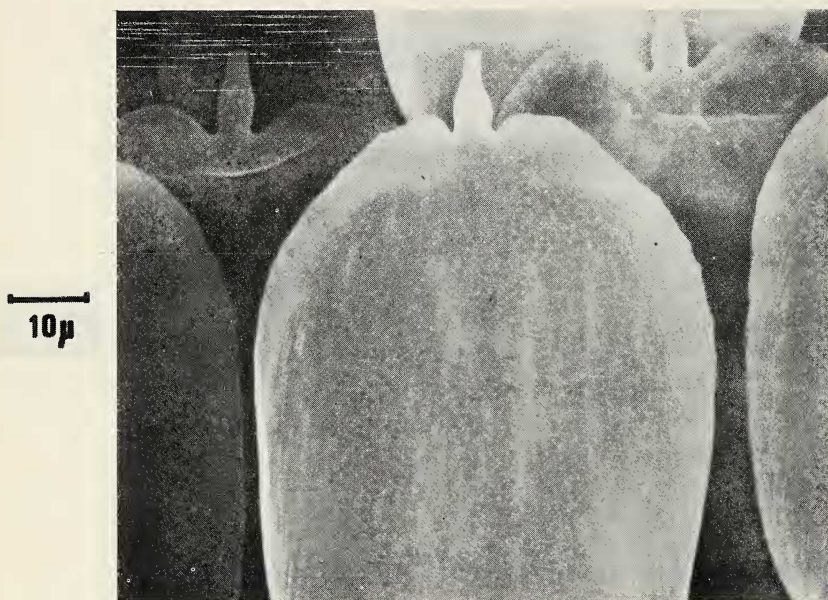
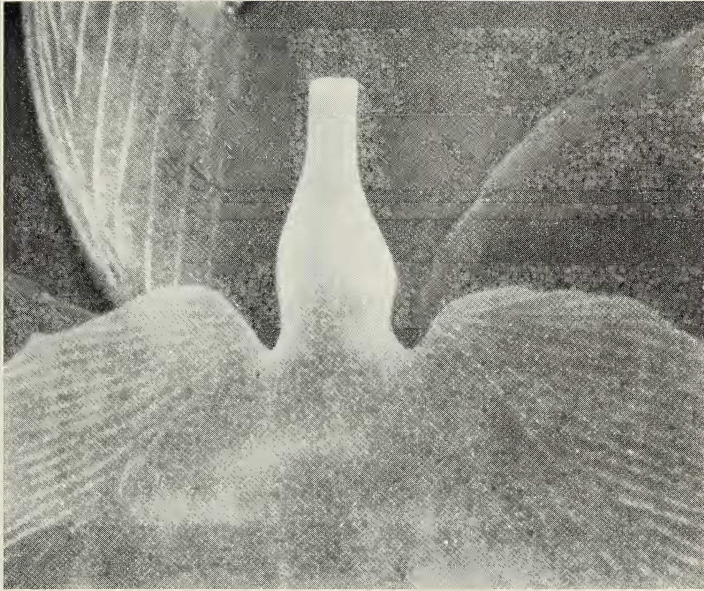


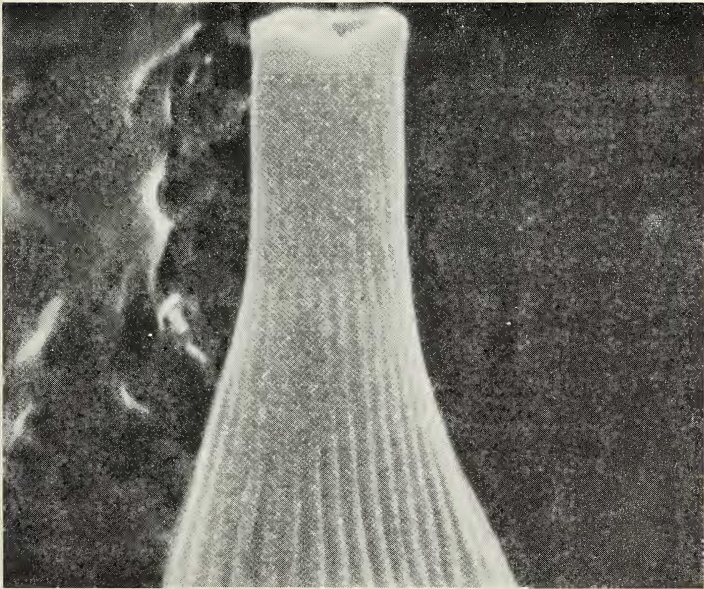
Fig. 4a.—Under surface, orange scales.

The orange color was removed completely by dipping a wing first in 95% ethanol and then for only 20 seconds in 20% aqueous ammonia (the color was transferred to the solution as the pteridines were dissolved as their ammonium salts). Then the wing was dipped in water, then ethanol, and allowed to dry in the air. Photomicrographs of the washed scales are shown in Fig. 3. The treatment seemed to make no change in the upper-layer scales (Fig. 3b vs. Fig. 2b), but in the case of the under-layer scales the suspended particles appear to have been largely removed to give a more open network (Fig. 3c vs. Fig. 2c). Whether and to what extent the ellipsoidal particles are related to the color remains to be established.

The under surface of the orange scale shown in Fig. 4 appears to be without much detailed fine structure. There are no ribs except on the peduncle and the periphery of the scale. This observation suggests that the scale resembles a hollow pouch consisting of two significantly different sheets—a continuous bottom membrane and a cross-ribbed upper sheet which is more or less porous depending on the type of scale and its position on the wing.



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μ



1
μ

Figs. 4b, c.—Under surface, orange scales.

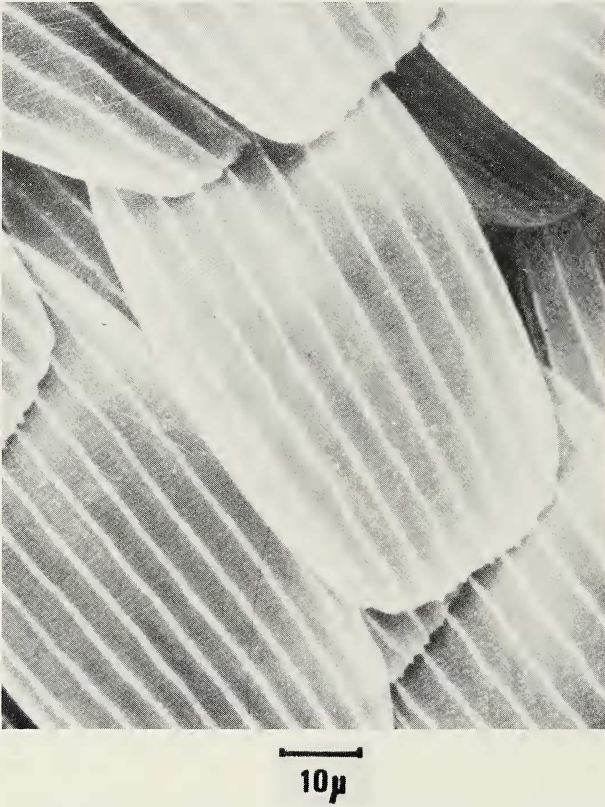
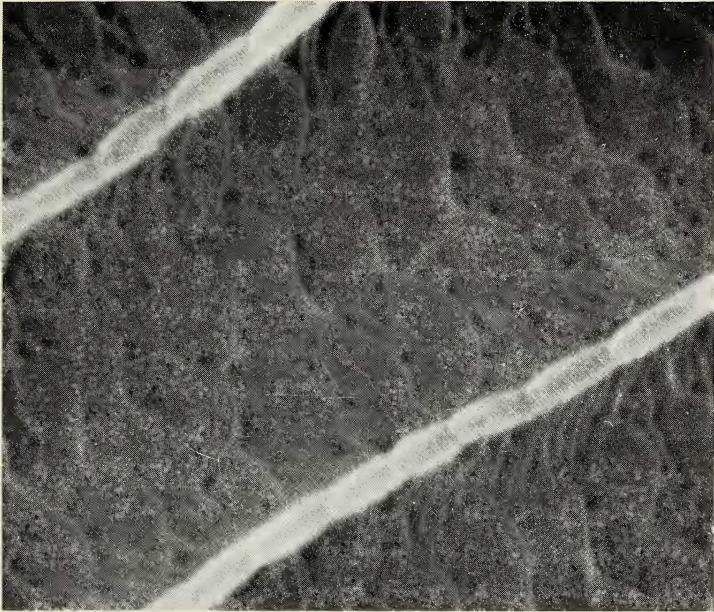


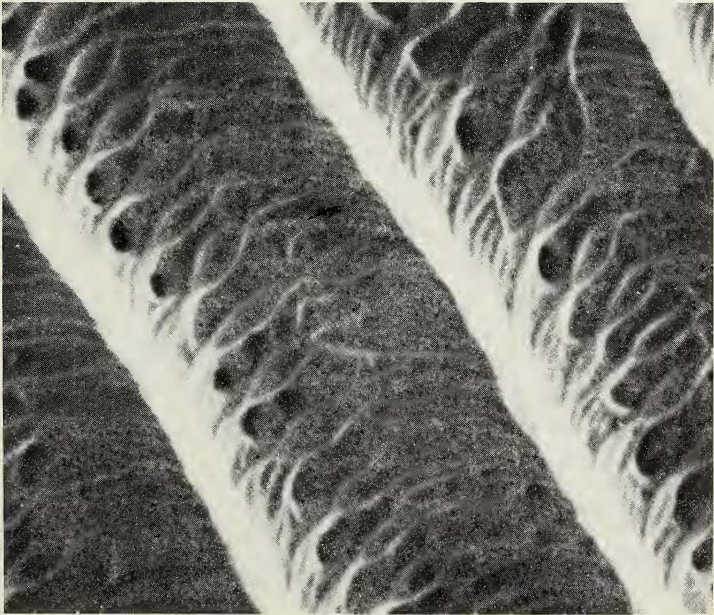
Fig. 5a.—Upper surface, black scales.

The fine structure of the upper surface of the black scales (Fig. 5) is strikingly different from that of the orange scales. The distance between the lengthwise ribs is approximately 3 to 6 microns as compared to 1.5 microns in the orange scales. Also, the trough-like material between the lengthwise ribs of the black scales displays intricate patterns which cannot be described as "cross-ribs" (Figs. 5b and c).

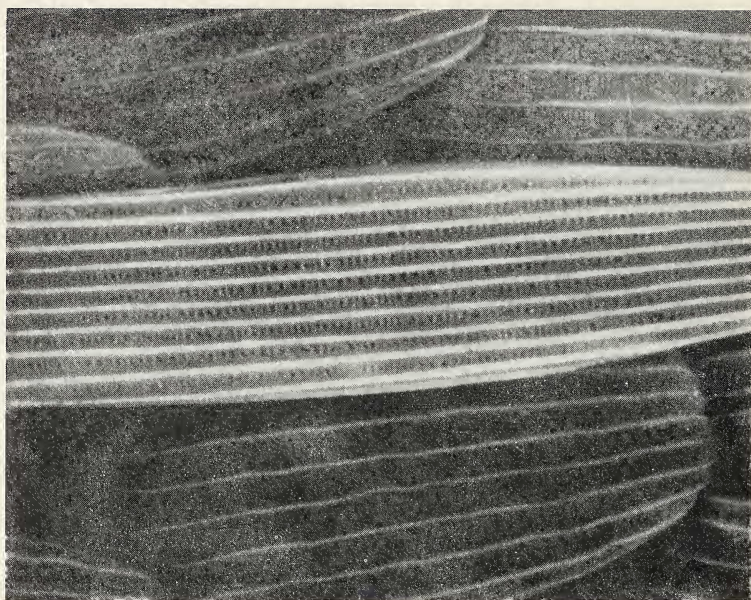
Interspersed among the black scales are brightly-colored yellow scales in which the distance between the lengthwise ribs is approximately 3 to 4 microns. The presence of cross-ribs, and particulate matter in some areas, is indicated (Fig. 6b).



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Figs. 5b, c.—Upper surface, black scales.



10 μ

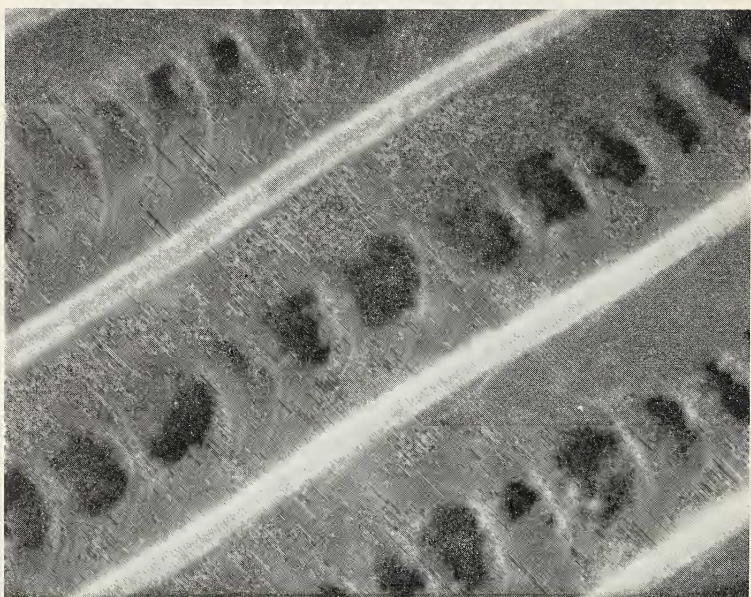


Fig. 6a, b.—Upper surface, yellow scales.

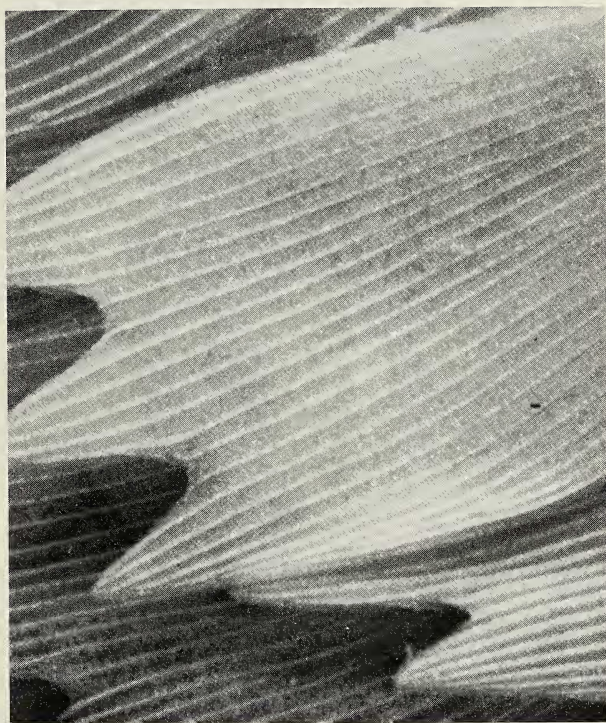
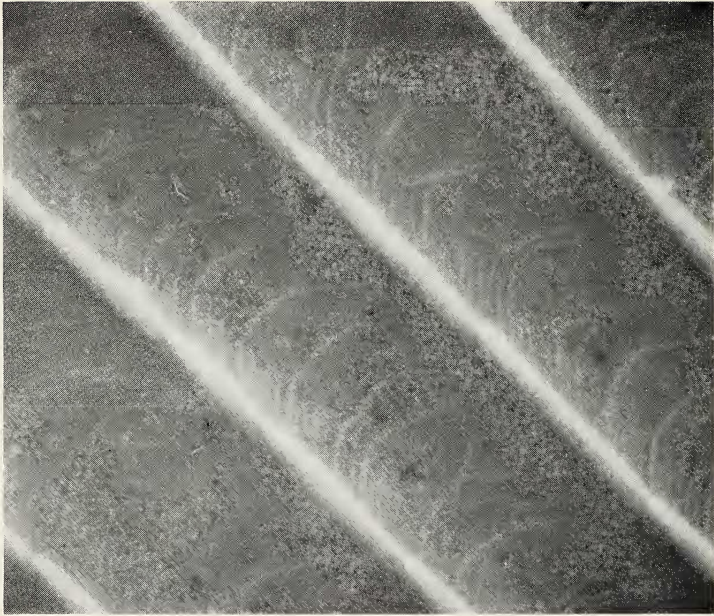
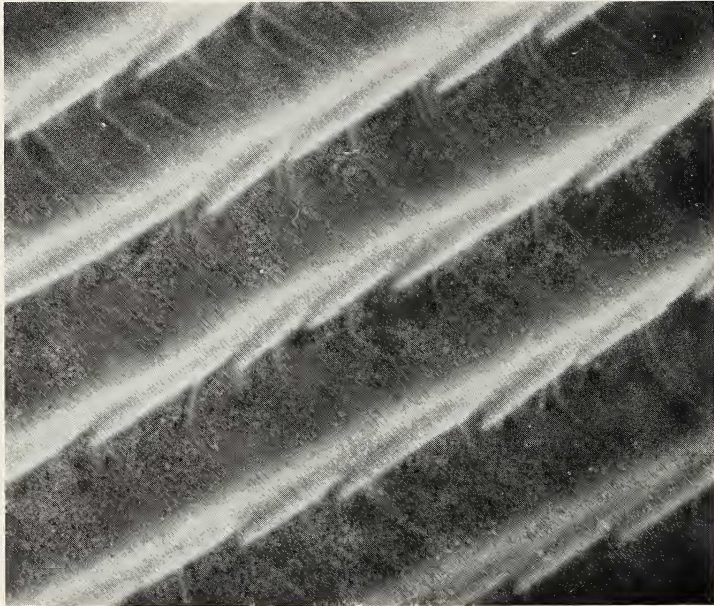


Fig. 7a.—Upper surface, pink fringe scales.

The final type of scales examined, the pink fringe scales, exhibit lengthwise ribs that are approximately 2 to 4 microns apart; the inter-rib distance varies from a minimum of about 2 microns at the basal region to a maximum of about 4 microns toward the tip of the scale. Tilting of the specimen showed clearly that the lengthwise ribs are composed of overlapping short narrow "scales" (Fig. 7c). The material connecting the lengthwise ribs in this case forms a continuous trough and appears to be supported by faintly-visible cross-ribs.



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Figs. 7b, c.—Upper surface, pink fringe scales.

SUMMARY

Fine structure varied greatly with color and position. The upper surface of an orange scale, cross-ribbed and perforated between the lengthwise ribs (1.5 microns apart), was strikingly different from the smooth and continuous lower surface as well as from the upper surface of a black scale, on which the ribs (5 to 6 microns apart) were connected by intricately-patterned "troughs". The peduncles (stems), as well as the scales themselves, appear hollow.

ACKNOWLEDGMENTS

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